PHCbi

You Don't Need Reproducible Research

UNTIL YOU DO.

If your work is successful in your lab, it should be successful on the other side of the world. That's why we engineer CO_2/O_2 incubators that deliver reproducible results, contamination free and equal to *in vivo* reality.

Minimize uncertainty with PHCbi brand products

PHC Corporation of North America

PHC Corporation of North America
1300 Michael Drive, Suite A, Wood Dale, IL 60191
Toll Free USA (800) 858-8442, Fax (630) 238-0074
www.phchd.com/us/biomedical

phchd.com/us/biomedical/cell-culture-incubators

PHC Corporation of North America is a subsidiary of PHC Holdings Corporation, Tokyo, Japan, a global leader in development, design and manufacturing of laboratory equipment for biopharmaceutical, life sciences, academic, healthcare and government markets.

Life Science Innovator Since 1966

Received: 7 November 2020 | Revised: 19 February 2021 | Accepted: 25 March 2021

DOI: 10.1002/bit.27778

ARTICLE

BIOTECHNOLOGY BIOENGINEERING WILEY

The effects of flux on the clearance of minute virus of mice during constant flux virus filtration

Rong Fan[1] | Fnu Namila[1] | Davar Sansongko[2] | S. Ranil Wickramasinghe[2] | Mi Jin[3] | Dharmesh Kanani[3] | Xianghong Qian[1]

[1]Department of Biomedical Engineering, University of Arkansas, Fayetteville, Arkansas, USA

[2]Department of Chemical Engineering, University of Arkansas, Fayetteville, Arkansas, USA

[3]Biologics CMC, Teva Branded Pharmaceutical Products R&D, Inc, West Chester, Pennsylvania, USA

Correspondence

Dharmesh Kanani, Teva Branded Pharmaceutical Products R&D, Inc, 400 Brandywine Pkwy, West Chester, PA 19380, USA.
Email: Dharmesh.Kanani@tevapharm.com.

Xianghong Qian, Department of Biomedical Engineering, University of Arkansas, Fayetteville, AR 72701, USA.
Email: xqian@uark.edu

Funding information

Teva Pharmaceutical Industries

Abstract

Constant flux virus filtration experiments were conducted to evaluate minute virus of mice retention behavior of four commercial virus filters for continuous bioprocessing applications. Fluxes chosen were guided by the Peclet number and the processing logistics as well as based on the filter characteristics. At the low flux condition of 5 $LM^{-2}H^{-1}$ (LMH) when diffusive force dominates, a significant breakthrough was observed for all the filtrate fractions for the filtration of a low fouling monoclonal antibody for three of the four filters. When both diffusive and convective forces are equally important at 40 LMH, virus breakthrough in buffer chase was observed only in one of the four filters investigated. When convective force dominates at 60 LMH or above, a high degree of virus clearance was observed for all three parvovirus filters investigated. Our work shed light on virus clearance during constant flux virus filtration for future continuous biomanufacturing.

KEYWORDS

constant flux filtration, continuous downstream processing, virus filtration

1 | INTRODUCTION

Mammalian cells are widely used during the production of therapeutic proteins, such as monoclonal antibodies (mAbs) and Fc-fusion proteins since their posttranslational modifications are more compatible with humans than those from prokaryote cells. However, mammalian cells inherently have an increased risk of virus contamination. It is therefore important to demonstrate adequate viral clearance with validation studies during downstream purification of these protein therapeutics (Guideline, 1997). Size-based virus filtration is often adopted toward the end of purification train to ensure adequate virus removal particularly for the non-enveloped small parvoviruses, which are difficult to clear with low pH or detergent inactivation methods.

Currently, downstream purification of biopharmaceuticals is largely operated under the batch mode. Continuous bioprocessing (CP) has significant potential advantages with improved productivity, better product quality, and a smaller footprint (Zydney, 2016). Even though CP has already been implemented during upstream production, for example, using a perfusion bioreactor, there are challenges for its adoption during downstream processing (Zydney, 2016). One of the challenges is to conduct virus filtration continuously. Virus filtration is routinely conducted under the normal filtration mode at constant pressure (Sofer et al., 2005) to maximize the throughput without violating the pressure rating of equipment or filtration module. Virus particles are rejected during viral filtration primarily via size exclusion. Commercial virus filters typically have a defined product throughput and also an upper limit on virus loading. Viral clearance may become inadequate if these limits are exceeded. Virus filtration is further complicated by the potential fouling of the membranes which could lead to compromised filter performance and reduced productivity. Virus filtration is currently targeted to achieve certain product throughput during a fixed amount of time such as during an 8-h shift (Wickramasinghe et al., 2010). Virus filtration validation studies are often performed using a scale-down model to achieve viral clearance targeting more than four logs of reduction value (LRV) while maximizing product throughput (Lute et al., 2007). Continuous downstream processing requires a consistent operation mode for virus filtration and the other chromatographic purification steps including protein A capturing step and cation or anion exchange chromatographic polishing steps (Bohonak et al., 2020; Kozlov et al., 2018; Shamashkin et al., 2013). One strategy is to conduct virus filtration at constant flux mode in line with other chromatographic operation modes. However, there is little knowledge of the performance of virus filters at low-pressure constant flux mode with regard to capacity and virus retention. Virus breakthrough or permeation through the virus filters is very complex and not well understood even at the commonly adopted constant pressure filtration mode. The effects of solution and operation conditions such as filtration flux and process disruption or pressure release on the retention of virus particles remain to be an active area of investigation.

An earlier study (Hongo-Hirasaki et al., 2010) investigated that solution conditions on virus filtration with one of the commercial parvovirus filters operated at a constant pressure recommended by the manufacturer and found that solution pH, buffer, and ionic strength affect the filtration performance of IgG feed spiked with porcine parvovirus (PPV). Filtration performance including throughput and flux was found to be strongly affected by the product–membrane interaction, less by the presence of dimers at the feed concentration range between 5 and 50 g/L. However, PPV retention was not affected by the range of solution conditions and product concentration studied. More recently, the effects of operating pressure on virus filtration were investigated for two commercial virus filters (Strauss et al., 2017) as it remains uncertain whether high pressure or low pressure represents the worst-case scenario for virus breakthrough. Optimal operating pressure ranges were identified for a range of solution conditions for minute virus of mice (MVM) retention. It was found that below certain operating pressure specific to each virus filter, MVM breakthrough could occur and the LRV could be affected by the solution condition. Moreover, MVM clearance is found to be product and filter-dependent. Recent studies (Namila et al., 2019; Stuckey et al., 2014) show that virus retention for one of the commercial filters is affected by solution conditions and is product-dependent. Earlier studies (Dishari et al., 2015; LaCasse et al., 2013, 2016; Willkommen et al., 2013; Woods & Zydney, 2014) have investigated the effects of process interruption or pressure release on virus retention during virus filtration of several commercial virus filters. These studies have indicated that flow disruption or pressure release could lead to the breakthrough of virus particles in the filtrate and a reduction in LRV.

The mechanisms for virus migration through the pores of the membrane were investigated at different filtration pressures for one of the virus filters (Yamamoto et al., 2014). Results indicate that back-diffusion at low-pressure and low flux conditions could lead to the permeation of virus particles through the filter. On the other hand, at high filtration flux conditions, hydrodynamic force dominates leading to the entrapment of the virus particles inside the filter. The flux ranges dominated by diffusion or convection can be estimated using Peclet number $Pe = \frac{ud_{virus}}{D}$, where u is the flow velocity, d_{virus} is the average diameter of the virus particle and D is the particle diffusion coefficient. Particle migration is dominated by hydrodynamic force when $Pe \gg 1$ whereas it is dominated by Brownian motion when $Pe \ll 1$. Its complementary modeling study also supports that virus particles tend to migrate more into the filter at low-pressure low flux conditions.

Here, this hypothesis is tested and mechanisms for virus breakthrough at low-pressure low flux conditions investigated. More specifically, virus filtrations are performed at low fluxes where diffusive Brownian motion dominates as well as at high fluxes where hydrodynamics convective force dominates for four commercially available filters. To differentiate the low flux caused by membrane fouling (Namila et al., 2019) and low flux due to a low operating pressure, low-fouling mAb feed solutions spiked by MVM particles are investigated.

The estimated transition filtration flux when Pe ≈ 1 could be estimated based on the diffusion coefficient PPV particles from earlier studies (Yamamoto et al., 2014). The effective diffusion coefficient was estimated to be ~2×10^{-13} m^2/s for PPV in buffer with an average diameter of 21 nm. Since the average MVM particle diameter is ~20–22 nm similar to the PPV particles, the same estimated diffusion coefficient D of 2×10^{-13} m^2/s can be used for MVM in buffer solution to determine the flux ranges where different forces dominate. As a result, the estimated flow velocity u is about ~10^{-5} m/s when Pe is 1, which can be translated into a flux value of ~36 LMH. Therefore, virus filtrations for flux values well below (5–15 LMH), at around (30–50 LMH), and above (60–400 LMH) this transition flux value were performed to investigate virus retention behavior.

2 | MATERIALS AND METHODS

2.1 | Preparation of feed stream

The protein mAb provided by TEVA Pharmaceuticals is an IgG_4 type of monoclonal antibody (mAb) expressed in CHO. The protein was formulated at pH 4.5 and 2 mS/cm conductivity and was shipped frozen in a 5 L bottle on dry ice at a concentration of 11.82 g/L. The protein was divided into 500 ml aliquots in sterile Nalgene PETG bottles and kept frozen at −80°C until use. The concentration of the protein was determined by measuring the absorbance at 280 nm with a spectrophotometer, Genesys 10 UV Scanning System (Thermo Fisher Scientific) with a VWR quartz spectrophotometer cuvette (path length 1 cm). The pH of the solution was determined with the pH 500 Economy Benchtop Meter (Oakton Instruments). The conductivity of the feed was monitored by the symphony SP70C. The Thermo Scientific™ Orion™ Star A215 pH and Conductivity Benchtop Meter was also used to measure the pH and conductivity occasionally.

The frozen protein solution was thawed in a water bath at room temperature (20–22°C). Before virus filtration experiments, the protein feed was pretreated with a Millistak+® Depth Filter in µPod® format with X0HC media series (area: 23 cm^2, cat #:

MX0HC23CL3) and an Optiscale® Capsule Polysep® II 1.0/0.2 µm (area: 17.7 cm^2, cat#: SGW3A47HH3; MilliporeSigma) using a Masterflex peristaltic pump (Cole-Parmer) to remove process- and/or product-related impurities, which can have significant impact on the volumetric capacity of virus filters. The pH of the protein solution was first adjusted from 4.5 to 7.5 with 1 M Tris. X0HC was vented with DI water at a 6 ml/min flow rate followed by flushing with DI water at a 23 ml/min flow rate for 10–15 min according to the manufacturer's recommendation. A Polysep II was then connected to the downstream of the X0HC and primed with DI water with a flow rate of 5 ml/min. Once the Polysep II was vented, the entire prefiltration train was flushed with DI water for 20 min. The same procedure was repeated with the equilibration (EQ) buffer of 25 mM sodium acetate buffer at pH 7.5. The EQ buffer was then replaced with a 500 ml protein sample and filtered at 2.5 ml/min. Due to the 25–40 ml hold-up volume of the prefiltration train, the first 20 ml of the sample was discarded into the waste container. At the end of prefiltration, the sample was chased with 30 ml of EQ buffer at 3 ml/min. After prefiltration, the feed solution was handled gently and stored at 4°C. Virus filtration experiments were performed within 2–4 days after prefiltration.

Before each virus filtration experiment, the aforementioned pretreated protein feed solutions were titrated from pH 7.5 to pH 5.5 with 10% acetic acid. Its salt concentration was adjusted to 135 mM with 3 M NaCl. Both the 10% acetic acid and the 3 M NaCl solutions were added slowly dropwise with gentle mixing. The feed solutions with pH 5.5 and 135 mM NaCl were then filtered with a cation exchange adsorptive prefilter, Viresolve® Pro Micro Shield (MilliporeSigma), to remove any additional aggregates. The VPro Shield was first flushed with DI water and equilibration buffer (25 mM sodium acetate buffer with 135 mM NaCl) at 15 psi. The protein feed proteins were subsequently filtered at 2.5–3 ml/min.

Chemicals used to prepare the buffer solutions are listed below: Sodium Chloride (Biotechnology Grade) from VWR Life Science, Tris base (Biotechnology Grade) from G-BioSciences, OmniPur Sodium Acetate Trihydrate (Molecular Biology Grade) from EMD Millipore, and Glacial Acetic Acid from Sigma-Aldrich (MilliporeSigma). DI water used for water flush and the buffers (for equilibration and titration) were filtered with 0.2 µm bottle-top vacuum filters.

2.2 | Virus production and purification

The MVM (ATCC® VR1346™) virus stock and the corresponding host mammalian cell, A9 (ATCC® CCL-1.4™), were purchased from American Type Culture Collection. The high titer high purity MVM virus stocks were produced and purified in-house following a published patent (Asher et al., 2017). A9 cells were grown in T175 CELLSTAR® cell culture flasks with cell growth medium with high glucose DMEM (Sigma-Aldrich) medium supplemented with 10% FBS (Gibco™, Thermo Fisher Scientific), 1% L-Glutamine from Sigma-Aldrich, and 1% sodium pyruvate from Corning®. After reaching a full confluency, the cell growing medium was aspirated. The A9 cell monolayer was then inoculated with MVM stock at a multiplicity of infection (MOI) around 2.5. Thereafter, 5 ml of MVM production medium with Gibco™ advanced DMEM supplemented with 1% FBS, 4% L-glutamine, and 1% Corning® non-essential amino acid (NEAA) was added into the flasks. Cells were inoculated in a cell culture incubator set at 37°C for 1.5 h to allow their absorption of MVM virus particles. Afterward, 35 ml of the same MVM production medium was added into the flask and kept in the cell culture incubator. Three days after inoculation, the MVM production medium containing 1% FBS was replaced with serum-free MVM production medium. After 18–21 days of inoculation, the cell culture medium with cell lysates was harvested into a 50 ml tube. Clarification was done with centrifugation followed by sterile filtration methods. An Amicon® Ultra-15 centrifuge filter device 100k (Millipore Sigma) was used to buffer exchange the MVM from the clarified medium into 10 ml of TNE buffer (10 mM Tris, 150 mM NaCl, 1 mM EDTA). Further purification and concentration were done by ultracentrifugation at 77,100*g* for 4 h at 4°C using an Optima XPN-100 Ultracentrifuge with an SW 41 Ti swinging-bucket rotor (Beckman Coulter). The supernatant was removed, and the pellets were soaked in 2 ml of TNE buffer and stored at 2–8°C for 2 days. Then, the MVM stock solution was sterile filtered and stored at −80°C until use.

2.3 | Constant flux viral filtration

Virus filtrations were conducted using commercial virus filters, which were designated as Filter N, Filter B, Filter V, and Filter H in this study. The pre-filtration with VPro shield as well as the visual leak tests (VLTs) for virus Filter N and Filter B were done at constant pressure using a Planova™ reservoir (Asahi Kasei) pressurized with industrial nitrogen gas. The prefiltered protein solution was spiked with MVM virus then filtered with a 0.2 μm bottle top filter. The constant flux virus filtrations were done with a NE-1000 series of syringe pump (New Era Pump Systems Inc) at lower flow rates and with ÄKTA FPLC system (GE Healthcare Life Sciences) at higher flow rates. Filters were first flushed with DI water and EQ buffer. 150 L/m^2 MVM spiked feed solution was loaded onto each virus filter and the filtrate was collected into a total of three fractions for each filtration. A Mettler Toledo scale connected to a BalanceLink software was used to record the cumulative weight of the filtrate every minute. A 15 min of process interruption was introduced after product filtration, followed by a buffer chase with 30 ml of EQ buffer in each experiment.

2.4 | MVM titration and clearance

The targeted feed titer for MVM is 9 logs/ml (qPCR) for all the feed conditions. The details on our MVM qPCR assay were described previously (Namila et al., 2019). After the filtration, virus titers in the feed, filtrate and buffer chase were determined by Tissue Culture Infectious Dose 50 ($TCID_{50}$) assay. The indicator cell, NB324K, was gifted by Peter Tattersall at Yale University. NB324K cells were grown in a T75 CELLSTAR® flask to full confluency with high glucose DMEM medium containing 10% FBS, 1% L-Glutamine, 1% NEAA, and 0.1 U/ml Penicillin/streptomycin. The cells were then washed with DPBS (Sigma-Aldrich) and trypsinized with 0.25% Trypsin-EDTA (TE) for 3–5 min in a 37°C incubator. A total of 5 ml freshly prepared complete medium was added into the flask to stop the enzyme reaction. The cell suspension was transferred into a 15 ml centrifuge tube and centrifuged at 130*g* for 5 min. The supernatant was discarded, and the cells were resuspended in 5 ml of fresh complete medium. After the cell density was determined with a standard hemocytometer and trypan blue, a required number of NB324K cells were suspended in the seeding medium then seeded onto a Nunclon™ Delta Surface 96-well plate (Thermo Fisher Scientific) at a density of 3000 cells/well with 100 µl/well. The composition of the seeding medium was: high glucose DMEM medium with 2% FBS, 1% L-Glutamine, 1% NEAA, and 0.1 U/ml penicillin/streptomycin. To avoid evaporation, in each 96-well plate, only the 60 wells in the middle were seeded with cells while the surrounding wells were filled with sterile DI water. The cells were incubated at 37°C with 5% CO_2 overnight. On the second day, when the desired confluency of 20%–50% was reached, a five serial tenfold dilution was made to each virus-containing sample with the seeding medium. Two replicates were done for each sample on one 96-well plate. Each dilution was inoculated onto six wells (one column) at 100 µl/well. Negative control wells were inoculated with the same seeding medium. Plates were returned to the cell culture incubator and kept for 10 days. On Day 10, each well was inspected under a microscope for CPE. The Spearman–Kärber method (Dougherty, 1964) was used to calculate $TCID_{50}$ titer. The 95% confidence limit, C ($C = \pm 2\ S_e$), was determined for each assay where S_e is the standard deviation.

When the virus titer in the filtrate is lower than the detection limit of the $TCID_{50}$ assay, a large volume plating (LVP) assay was used for determining virus titer. Here, virus-containing samples were diluted three times and then inoculated into all the 96 wells with 200 µl/well. The virus titer was calculated based on the formula if virus-induced changes are observed in only a few wells of the LVP (<15% of all wells; Gavasane et al., 2013). If no virus-induced changes are observed for a sample, the virus titer is determined by the Poisson distribution at the 95% confidence limits (CPMP, 1997). LRV is a commonly used term for characterization of virus clearance and it refers to the difference between the total viral load in feed and product pool.

$$LRV = Log_{10}\frac{C_{fd\times}V_{fd}}{C \times V} \quad (1)$$

where C_{fd} is the virus titer in the feed, V_{fd} is the volume of the product processed through the virus filtration step; C and V are the virus titer and the volume of the permeate, respectively.

2.5 | Experimental design

The main objective of this study is to understand the impact of flux on virus breakthrough as well as to achieve some understanding of process design flexibility in the context of continuous manufacturing. In a typical batch process, virus filtration is sized to process the entire batch in one cycle. In the continuous manufacturing process, the perfusion cell culture and chromatography are operated at a constant flow rate. Therefore, it is preferred to operate virus filtration at constant flux from mass balance perspective. Considering the duration of perfusion cell culture, it is desirable to perform virus filtration in multiple cycles. Considering the virus filters are validated for certain fixed capacity, the number of cycles is dictated by operating flux and perfusion cell culture duration. Table 1 shows a few scenarios of operating flux for a range of filter capacity and process time. From process design perspective, a filter that can be operated over a range of flux without compromising its virus retention ability provides flexibility in sizing virus filtration operation.

Here, the effects of filtration flux and throughput on the retention of MVM were evaluated at constant flux filtration mode with and without the presence of a protein product. Four commercially available virus filters (Filters N, B, V, and H) were selected for the investigation. The filter materials of these four virus filters and manufacturers' recommended operating condition ranges were listed in Table 2.

During the first set of studies, buffer only filtrations spiked with 9.0 logs/ml (qPCR) were evaluated for Filter N at pH 4, 5.5, and 7 with and without 135 mM NaCl at constant pressure mode (10 psi). The buffer condition was chosen to be 25 mM Tris-acetate as this is a commonly used buffer system during downstream purification of protein therapeutics. Filter N has a relatively low filtration flux range due to its low-pressure rating (up to 14 psi) but with a high resistance to fouling. The first goal is to find a solution condition where virus breakthrough is more likely to occur. For that purpose, a high feed virus titer at 9.0 logs/ml (qPCR) and a throughput at 150 L/m^2 were chosen for the investigation for Filter N. Table 3 list the conditions for buffer only filtration with Filter N. Three equal fractions of the permeate at increasing viral load were collected for the $TCID_{50}$ assay for MVM titer determination to understand virus breakthrough behavior at the beginning, middle and end of filtration.

Once an appropriate buffer condition was identified where virus breakthrough could be observed for Filter N, viral filtration experiments for all four filters operated at constant flux with the identified feed buffer condition were performed. As discussed previously, three different flux levels will be investigated for MVM retention during virus filtration representing the flux well below, and well above the Peclet number of 1. For MVM, the flux is about 36 LMH when Pe is 1.

TABLE 1 Flux value required for filtering a certain amount of product within 8, 24, 48, or 72 h operated at constant flux mode

		Filter capacity (L/m^2)			
Flux (LMH)	150	250	1000	4000	
Time (h)	8	19	31	125	500
	24	6	10	42	167
	48	NP	5	21	83
	72	NP	NP	14	56

Abbreviation: NP, not practical.

TABLE 2 The characteristic of virus filters used in the study

Virus filter	Filter area (m^2)	Typical operation pressure (psi)	Filtration flux at typical operating pressure (LMH)	Material and module
Filter N	0.001	10–12	30–50	Cuprammonium regenerated cellulose hollow fiber
Filter B	0.001	30	60–100	Hydrophilized PVDF Hollow fiber
Filter V	0.00031	30	350–450	Hydrophilized PES Flat sheet
Filter H	0.0005	30	250–350	Modified PES Hollow fiber

A flux value of 5 LMH represents the low flux condition when diffusive force dominates the migration of virus particles with Pe $\ll$1. In the context of continuous processing, the low flux scenario reduces the number of cycles as virus filtration is sized for low-pressure long duration operation. This would also eliminate the frequent filter set-up and the need for pre-use and post-use filter integrity testing for each cycle. A flux value of 40 LMH represents the condition when both diffusion and convection play a role in virus transport with Pe ~ 1. This is the normal filtration flux for Filter N, but significantly lower flux for Filters H, B, and V. A flux value much higher than 40 LMH represents the condition when convective force dominates for the MVM particle movement in the filter with Pe $\gg$1. A high filtration flux of 80 LMH was investigated for Filter B, a flux of 300 LMH was evaluated for Filter H and a flux of 400 LMH was evaluated for Filter V, all of which are typical operating fluxes for the respective filters in a typical constant pressure batch process. Besides the filtration flux, different filter capacities (25 L/m^2 for Filter N and B, 50 L/m^2 for Filter V) or high throughputs (150 L/m^2) were studied for the filters to understand the impact of total viral load on virus breakthrough during buffer chase. The targeted MVM titer was again at 9.0 logs/ml (qPCR). Table 4 shows the feed volumes for the selected filter sizes and times needed for constant flux filtrations at 5 and 40 LMH as well as at their normal filtration fluxes.

Finally, viral filtration experiments with feed solutions containing 10 g/L of mAb from Teva Pharmaceutical were conducted for all four filters at fluxes of 5 LMH (Filters N, B, V, and H), 40 LMH (Filters N, B, V, and H), 80 LMH (Filter B), 300 LMH (Filter H), and 400 LMH (Filter V), the same as those conducted for the buffer only experiments. The throughout for all the filters was set at 150 L/m^2. The targeted MVM titer in the feed was 8 logs/ml (qPCR). The one-log reduction in the targeted MVM feed titer arises from the fact that virus breakthrough is more likely in the presence of protein products (Bolton et al., 2005). The filtrate was collected in three fractions. Having different volumetric throughputs or collecting filtrate in fractions was to evaluate the effect of total viral load on virus retention of the filters. A process interruption with complete depressurization was introduced after product filtration and before 30 ml buffer chase in all runs. Table 5 lists the constant flux runs with and without the protein.

TABLE 3 Solution conditions tested for Filter N at constant pressure mode (10 psi) with 20 mM acetate buffer only spiked with 9.0 logs/ml MVM (qPCR)

Virus filter	Load (L/m^2)	Targeted virus titer (logs, qPCR)	pH	NaCl (mM)	
Filter N	150	9	4	0	135
			5.5		
			7.5		

3 | RESULTS AND DISCUSSIONS

3.1 | Constant flux filtration of MVM spiked buffer feed solutions

The effect of the solution condition on MVM clearance was first evaluated with Filter N at 10 psi in constant pressure mode. Filtrations were conducted for buffer feed solutions containing 25 mM Tris-acetate at pH 4.0, 5.5, and 7.5 with or without 135 mM NaCl. The feed buffers were spiked with 9.0 logs/ml (qPCR) MVM. The filtration experiments were

TABLE 4 Feed volumes and filtration times needed at different flux levels for each of the four filters investigated at 150 L/m^2 capacity

		Filtration time (h) Constant flux value (LMH)				
Filter type	Feed volume (ml)	5	40	80	300	400
Filter V	47.5	30.4	3.8	–	–	0.4
Filter N	150	30.1	3.7	–	–	–
Filter B	150	30.1	3.7	1.9	–	–
Filter H	75	29.8	3.7	–	0.5	–

TABLE 5 Experimental design of the constant flux virus filtration runs for the four filters

Flux (LMH)	Filter	Throughput (L/m^2)
		150 (++)
	Filter N	25 (+−)
		150 (++)
	Filter B	25 (+−)
		150 (++)
	Filter H	150 (++)
40	Filter V	150 (++)
	Filter N	150 (++)
	Filter B	150 (++)
40	Filter H	150 (−+)
80	Filter B	150 (++)
300	Filter H	150 (−+)
400	Filter V	150 (++)

Note: The two symbols (+ or −) in the bracket indicate the sequential status of buffer filtration only and filtration in the presence of protein product.

performed with a total of 150 ml feed solutions at a pressure of 10 psi as recommended by the manufacturer. Three fractions of the filtrate were collected. In addition, a 15 min pause followed by 30 ml of buffer chase was performed for all the runs. The filtration fluxes (not shown) for all the runs were between 40 and 50 LMH with only a slight decay of ~10% indicating the MVM spiking did not cause much fouling on the membrane. Figure 1 plots MVM infectivity titers measured by $TCID_{50}$ in the feed buffer, three filtrate fractions, and buffer chase. It can be seen that only two conditions demonstrate minor virus breakthrough. At pH 5.5 with 135 mM NaCl, virus breakthrough in fraction 3 and buffer chase was observed. At pH 7 with 0 mM NaCl, virus slight breakthrough was observed for all three fractions, but not in the buffer chase. The overall LRVs (Table S1) for all the conditions are above 4 indicating that Filter N is robust for viral clearance for the Tris-acetate buffer spiked with an average ~6.5 logs/ml ($TCID_{50}$) MVM.

For the feed with pH 7.5 and 0 mM NaCl, the spiked MVM titer is the highest at 6.83 logs/ml ($TCID_{50}$) as shown in Table S1 among all the runs. It is known that MVM breakthrough could occur for Filter N when the viral loading capacity has reached. Even though the targeted MVM titers were supposed to be the same for all the runs, there is always some variation in MVM feed titer introduced by the spiking process for the individual run. The slight breakthrough observed at this condition could be due to the higher feed titer spiked. On the other hand, for the buffer condition at pH 5.5 with 135 mM NaCl, the spiked feed titer at 6.33 logs/ml ($TCID_{50}$) was below four of the six runs, yet virus breakthrough at third fraction and buffer chase was observed indicating that this could potentially be the buffer condition prone for virus breakthrough. As a result, pH 5.5 with 135 mM NaCl was chosen to be the buffer condition for the subsequent studies. The reason why the breakthrough occurs at pH 5.5 with 135 mM NaCl in 25 mM Tris-acetate buffer is not entirely clear. There could be several possible reasons. Since MVM has a pH of 6.0, its surface charge is close to neutral at pH 5.5 leading to a slight reduction in its size. Moreover, the electrostatic interaction is weaker at feed condition with 135 mM NaCl compared to the feed condition with 0 mM NaCl resulting in a reduction in its interaction with the filter surface. Reduction in its size and weaker interaction with the filter could potentially increase its chance of migrating

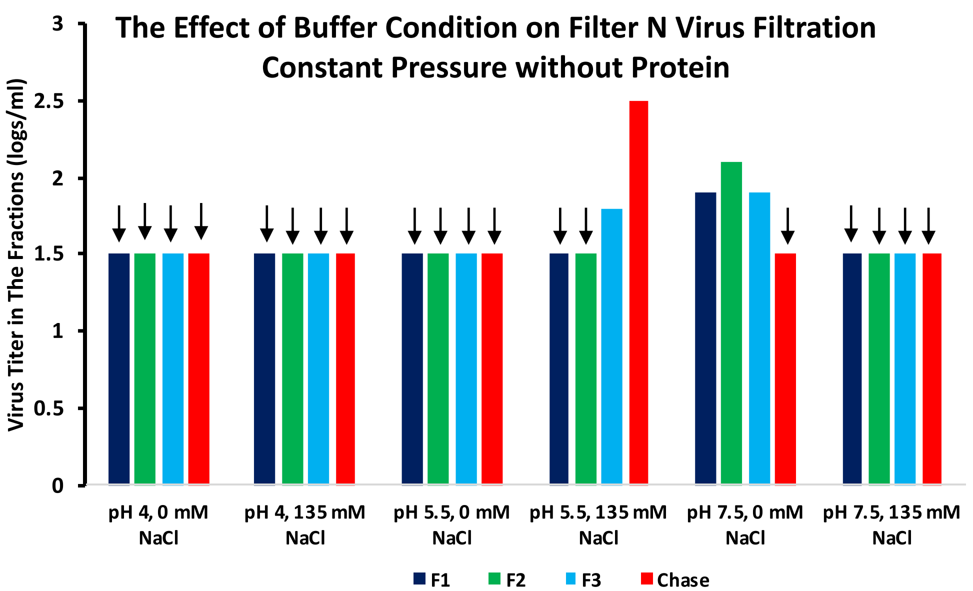

FIGURE 1 MVM titers ($TCID_{50}$) of buffer (25 mM Tris-acetate) only virus filtration runs with Filter N. Shown are the MVM titers of three filtrate fractions and buffer chase. The average feed titer is ~6.5 logs/ml ($TCID_{50}$). The arrow symbol indicates the virus titer is below the detection limit of 1.5 logs/ml

through the filter during the filtration process. However, virus retention and breakthrough during viral filtration is complex and currently not well understood.

After the buffer condition where virus breakthrough is more likely to occur was selected at constant pressure filtration with Filter N, constant flux filtration experiments were performed for all four filters at three different flux levels and at low and high throughputs. Since these are spiked buffer only filtrations, only very slight pressure increase over the course of filtration was observed indicating low fouling on the virus filters. At 5 LMH filtration flux and with low throughput conditions (25 L/m^2 for Filter N and B, 50 L/m^2 for Filter V), only one fraction of the filtrate was collected. For all other conditions, three equal fractions were collected for the titer assay. Table 6 exhibits the MVM titers in the feed, fractions, and buffer chase for the investigation. The overall LRVs were also shown. For all the runs, small virus breakthrough was only observed in the filtrate and buffer chase for Filter V at 5 LMH and 50 L/m^2 throughput run. The filtrate fraction was collected over a 10 h period. To confirm that this was not contamination or due to the uncertainty in the assay, additional two runs at this condition were performed. In one of the runs, no virus breakthrough was observed. However, virus breakthrough was again observed in the filtrate and buffer chase at low levels. It seems that extremely low flux for Filter V can lead to virus breakthrough. At a very low flux of 5 LMH, convective flow is not as dominant compared to high flux conditions. Instead, diffusion plays a more important role. This allows virus particles that are previously trapped in smaller pores by convective flow to back diffuse and migrate to larger pores leading to the breakthrough of the virus particles in the filtrate. This can be often observed after process interruption in the buffer chase where low pressure hold leads to the back migration of the virus particles. To further validate that low flux can lead to virus breakthrough, one more repeat of Filter V at 5 LMH and 150 L/m^2 throughputs was performed. Even though no virus breakthrough was observed in the first run, it can be seen from Table 6 that virus breakthrough was observed in all three fractions as well as in buffer chase for the second run. When flux increases to 40 and 400 LMH, no virus breakthrough was observed for Filter V confirming that diffusion could indeed be an important factor for virus permeation through the membranes. For Filters N, B, and H, no virus breakthrough was observed at all the filtration fluxes and throughputs. This indicates that membrane structure may also play an important role in the retention of virus particles. For all four filters, the overall LRV is above 4 except for one run with Filter V. It is worth mentioning that at 5 LMH, the filtrate was collected over a period of 30 h for a filter capacity of 150 L/m^2 whereas at 40 LMH or higher, the run time was much shorter at ~4 h or less. Moreover, at a flux of 5 LMH, the filtration pressure is far below the manufacturer's recommended pressure range for all the filters.

3.2 | Constant flux filtration of protein feed solutions

The effects of filtration flux on viral filtration were investigated in the presence of protein product at constant flux mode. Virus filtration experiments were conducted for feed solutions containing 10 mg/ml mAb

TABLE 6 MVM titers ($TCID_{50}$) in the 25 mM Tris-acetate feed buffer, three fractions and buffer chase for buffer only virus filtration runs with Filters N, B, V, and H at constant flux mode. The overall LRVs are also shown

Flux (LMH)	Filter	Throughput (L/m^2)	Feed titer (logs/mL)	Virus titer for each fraction (logs/mL) F1	F2	F3	BC	LRV (logs)
5	**Filter V**	50	6.83	1.83	N/A		1.75	**4.82**
		50	6.09	≤1.50			≤1.50	≥4.39
		50	6.17	2.09			2.34	**3.76**
		150	6.25	≤1.50	≤1.50	≤1.50	≤1.50	≥4.67
		150	6.67	1.67	1.91	2.08	1.83	**4.68**
	Filter N	25	6.92	≤1.50	N/A		≤1.50	≥5.08
		150	6.75	≤1.50	≤1.50	≤1.50	≤1.50	≥5.17
	Filter B	25	6.75	≤1.50	N/A		≤1.50	≥4.91
		150	6.75	≤1.50	≤1.50	≤1.50	≤1.50	≥5.17
	Filter H	150	5.92	≤1.50	≤1.50	≤1.50	≤1.50	≥4.27
40	**Filter V**	150	6.75	≤1.50	≤1.50	≤1.50	≤1.50	≥5.17
	Filter N	150	7.09	≤1.50	≤1.50	≤1.50	≤1.50	≥5.51
	Filter B	150	6.67	≤1.50	≤1.50	≤1.50	≤1.50	≥5.09
80	**Filter B**	150	6.75	≤1.50	≤1.50	≤1.50	≤1.50	≥5.17
400	**Filter V**	150	6.75	≤1.50	≤1.50	≤1.50	≤1.50	≥5.17

at the selected same buffer condition of 25 mM acetate buffer at pH 5.5 and with 135 mM NaCl at three flux levels. Similar to the spiked buffer filtrations, low filtration flux of 5 LMH was run for Filters V, N, B, and H. Viral filtrations were also performed at a medium filtration flux of 40 LMH for all four filters. Filtration at a flux of 80 LMH was run for Filter B. For Filter V, filtration at 400 LMH and for Filter H, filtration at 300 LMH were also performed. The throughput was fixed at 150 L/m^2 for all the filtration studies. Three filtrate fractions and one buffer chase after 15 min process interruption were collected for MVM titer determination. The mAb studied is a low fouling protein with no or only slight pressure increases over the course of filtration for all four filters.

Figure 2 shows the MVM titers in the feed, filtration fractions, and buffer chase with a constant filtration flux of 5 LMH. All titrations were conducted with TCID$_{50}$ assay. In addition, LVP assay was also performed for filtrate fractions and buffer chase of Filter B since no virus breakthrough was observed with TCID$_{50}$ assay. Since virus breakthrough was observed, repeat runs for Filters V and N were performed as shown in Figure 2. The repeat runs exhibit a similar trend for both filters. It can be seen that significant breakthrough was observed for Filters V, N, and H. The overall LRVs are below 4 logs with an average feed titer of ~5.5 log/ml (TCID$_{50}$; Table S2). On the other hand, breakthrough for Filter B was only observed in LVP for the last two fractions and buffer chase. The titer actually appears to be lower in fraction 2 (−0.98 logs) with breakthrough than in fraction 1 (≤−0.40 logs) without breakthrough due to different methods used for quantification. Filter B has an LRV of 5.9 logs (Table S2) indicating its robustness in MVM retention during filtration in the presence of a protein product. For Filter V in the presence of the mAb, MVM breakthrough becomes more severe with LRV reduced to below 4 logs. This again confirms that at very low constant flux of 5 LMH, trapped MVM particles in the smaller pores of the filter can back diffuse and move along the larger pores leading to virus breakthrough. Diffusion appears to play an important role in virus retention for Filter V particularly when longer filtration time is needed at such a low flux. An earlier study (Bohonak et al., 2020) shows that a low flux at 4.5 LMH did not lead to virus breakthrough. This is likely due to the fact that particle diffusion is restricted with a more viscose feed stream at a product titer of 50 g/L. This demonstrates the important role of diffusion plays on virus retention. Since transmembrane pressure remains more or less the same over the course of filtration, it appears that this mAb does not cause much fouling of the membrane. However, some of the protein or protein aggregates will be trapped in the membrane pores, particularly the smaller pores. This could force the virus particles to migrate along the larger pores. Combined with the diffusion migration mechanism, this leads to the permeation of the virus particles through the membrane. However, the normal operating pressure for Filter V is much higher, diffusion is not likely to dominate the migration of the virus particles under such high pressure. It is interesting that during spiked buffer filtration at 5 LMH, Filters N and H demonstrated no virus breakthrough with LRV reaching above 5 logs. However, in the presence of 10 g/L mAb, virus breakthrough was observed for both Filters N and H. It seems that the presence of mAb leads to virus breakthrough for these two filters. Protein and protein aggregates tend to compete for the pore pockets where virus particles could be trapped. Combined with the possibility of back diffusion, virus particle breakthrough is likely as observed here with two logs of reduction for LRV compared to the buffer only filtration. At low fouling conditions where some product molecules are adsorbed by the filter, the virus particles could be forced to go through the larger pores leading to their potential breakthrough. On the other hand, when fouling is significant, the filter and pore surfaces with accumulated foulants could effectively reject the virus particles leading to

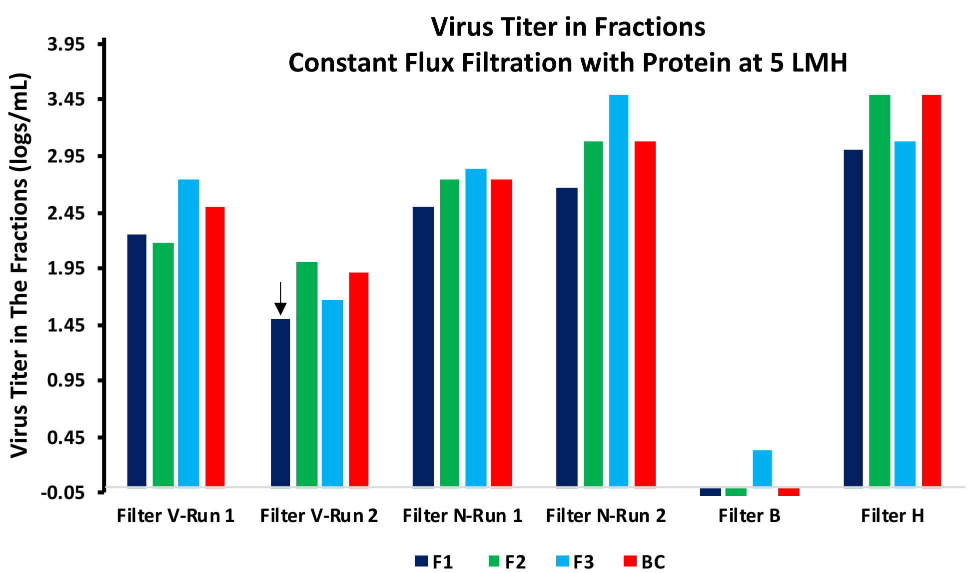

FIGURE 2 MVM titers of three filtrate fractions and buffer chase for the 5 LMH constant flux filtration of 10 mg/mL protein in 25 mM acetate buffer at pH 5.5 with 135 mM NaCl with four virus filters. LVP assay was performed for Filter B fractions. TCID$_{50}$ assay was performed for all other samples. The average feed titer is ~5.5 logs/mL (TCID$_{50}$). The arrow symbol indicates the virus titer is below the detection limit of 1.5 logs/mL (TCID$_{50}$)

TABLE 7 MVM titers in the feed, three filtration fractions, and buffer chase for the 40 LMH constant flux filtration of 10 mg/ml mAb in 25 mM acetate buffer at pH 5.5 with 135 mM NaCl with three virus filters. $TCID_{50}$ assay was performed for all the feed solutions as well as buffer chase from Filter N. LVP assay was performed for all other collected fractions

Virus filter	Feed titer (logs/mL)	Virus titer for each fraction (logs/mL)				LRV (logs)
		F1	F2	F3	BC	
Filter V	5.58	≤−0.49	≤−0.49	≤−0.49	≤−0.63	≥6.01
Filter N	6.08	≤−0.40	−0.68	0.16	2.42	**4.35**
Filter B	5.58	≤−0.40	≤−0.40	≤−0.40	≤−0.38	≥5.9
Filter H	5.33	≤−0.44	≤−0.44	≤−0.44	≤−0.43	≥5.62

an enhanced virus retention. However, the effect of fouling on virus retention remains to be controversial and will be the subject of a future study.

Filtration experiments were also performed for the four filters at constant flux of 40 LMH for the feed solutions containing 10 g/L mAb. Table 7 shows the titers of the feed, three fractions, and buffer chase for each filtration and the overall LRVs for Filters V, N, B, and H. No CPE was observed with the $TCID_{50}$ assay for the fractions and the buffer chase for Filters V, B, and H. As a result, LVP assay was performed for the samples with no CPE from $TCID_{50}$ assay. Filters V, B, and H demonstrate complete clearance with LRVs reaching ~6 logs. For Filter N, the LRV is also over 4 logs. Compared to the corresponding constant flux filtrations at 5 LMH, MVM clearance for the two conditions demonstrates substantial differences. Low flux at 5 LMH leads to significant virus particle permeation through the membranes whereas no breakthrough was observed at 40 LMH except for Filter N after process interruption. These results clearly indicate that low-pressure conditions where diffusion dominates could lead to virus breakthrough for Filters V, N, and H. In particular, a longer filtration time was required at low flux conditions to process the same throughput of 150 L/m^2. For Filter B, filtration flux does not appear to affect its viral clearance. LRVs of ~6 logs were reached at both 5 and 40 LMH. Maintaining a filtration flux above a certain threshold where convection dominates is critical for the performance of Filters V, N, and H.

At the normal operating pressure of 30 psi, fluxes of 80, 300, and 400 LMH can be reached for Filters B, H, and V, respectively. Virus filtration experiments were conducted for feed solutions containing 10 g/L of mAb at constant flux of 80, 300, and 400 LMH, respectively. For Filters B, H, and V, no CPE was observed for all the fractions and buffer chase with $TCID_{50}$ assay. LVP assay was conducted for all the fractions which resulted in complete clearance for all except the third fraction of Filter V. As shown in Table 8, the LRVs for filtrations with Filters B and V were at ~5.9 logs. The LRV for Filter H is ~5.4 logs due probably to the slightly lower feed titer. These results again indicate that high filtration flux or high pressure where convection dominates will lead to the improved retention of MVM particles. Very low filtration flux at low-pressure conditions could lead to significant virus breakthrough to the permeate. A recent study (David et al., 2019) where virus filtrations were performed for 72 h at 0.3 LMH is in agreement with our current study. It was found that low flux conditions could lead to a reduction of the virus retention of 20 L/m^2 bacteriophages spiked mAb feed solutions with some of the commercial filters. On the other hand, another recent 4.5 LMH constant flux virus filtration study (Bohonak et al., 2020) with a more viscous feed at high product titer did not exhibit MVM breakthrough. These studies support the important role of diffusion plays in virus filtration at low-pressure low flux conditions. However, high flux (>40 LMH) virus filters when operated at their normal pressure range are robust in their virus retention performances (Table 8).

To better understand the differences in filter performance as a function of filtration flux, the transmembrane pressures (TMP) $\bar{\Delta P}$ were monitored during the virus filtration experiments. Table 9 shows the approximate TMPs for three filters at different flux values for the virus filtration of the mAb feed solutions. The TMPs did not increase or only increased very slightly during the course of filtration experiments indicating that this mAb is a low fouling protein. It can be seen that Filter V has the lowest TMP, followed by Filter H at both 5 and 40 LMH conditions. Filters N and B have a much higher TMP at the corresponding fluxes. This indicate that membrane resistance is the lowest for Filter V and highest for Filter B based on Poiseuille's equation:

$$Flux = L_p\, \bar{\Delta P} \tag{2}$$

where L_p is the hydraulic conductance or permeability. Interestingly, virus retention properties of V and H as well as B and N are rather different despite their similarities in hydraulic conductance. This

TABLE 8 MVM titers in the feed, three filtration fractions and buffer chase for the 80 LMH (Filter B), 300 LMH (Filter H) and 400 LMH (Filter V) constant flux filtration of 10 mg/mL mAb in 25 mM acetate buffer at pH 5.5 with 135 mM NaCl with three virus filters. $TCID_{50}$ assay was performed for the feed solutions. LVP assay was performed for all the filtrate fractions and buffer chase

Flux (LMH)	Virus filter	Feed titer (logs/ml)	Virus titer for each fraction (logs/ml)				LRV (logs)
			F1	F2	F3	BC	
80	Filter B	5.58	≤−0.40	≤−0.40	≤−0.40	≤−0.38	≥5.91
300	Filter H	5.08	≤−0.44	≤−0.44	≤−0.44	≤−0.43	≥5.38
400	Filter V	5.58	≤−0.49	≤−0.49	−0.12	≤−0.63	**5.87**

TABLE 9 Approximate transmembrane pressure detected by pressure sensor and the corresponding permeability during virus filtration at different flux values for the four filters

	Transmembrane pressure (TMP, psi)/permeability (LMH/psi)				
Virus filter	5 LMH	40 LMH	80 LMH	300 LMH	400 LMH
Filter V	0.4/12.5	3/13.3	–	–	30/13.3
Filter N	1.5/3.3	12/3.3	–	–	–
Filter B	2.3/2.2	19/2.1	30/2.7	–	–
Filter H	0.5/10	4/10	–	27/11.1	–

does indicate that other properties of the filters may contribute to their differences in virus retention. For example, for Filters B and N, their pore sizes and pore size distributions could be somewhat different even though the membrane structures and thicknesses are similar to each other. This small difference in hydraulic conductance (2.2 LMH/psi for Filter B vs. 3.2 LMH/psi for Filter N) cannot explain the significant differences in their virus retention properties generally observed for these two filters. An earlier study (Giglia et al., 2015) indicates that a slight difference in pore size of the virus filters has a direct effect on virus retention and LRV.

4 | CONCLUSIONS

Constant flux virus filtration experiments were conducted to evaluate the virus retention behavior of four commercial virus filters for future continuous bioprocessing applications. Experiments were performed at three flux levels in the absence and presence of a low fouling monoclonal antibody. The fluxes chosen were based on the Peclet number, the processing logistics, and filter characteristics. It was found that at a very low flux of 5 LMH, when diffusive force dominates, MVM particles could breakthrough to the filtrate which leads to a slight reduction of the LRV for only one of the four filters in the absence of the protein. However, in the presence of a 10 g/L low-fouling mAb, three of the four filters exhibit severe MVM breakthrough at 5 LMH irrespective of the throughput. This can be explained by the competition of the protein molecules with the MVM particles for the cavity pockets where they are trapped and the back-diffusion of the virus particles through the larger pores. However, when flux increases to 40 LMH when both diffusive force and convective force play a role in virus particle migration, all four virus filters achieved an LRV of more than 4 logs with only Filter N exhibiting a slight breakthrough. At even higher flux values of 80, 300, and 400 LMH for Filters B, H, and V, respectively, when convective force dominates, high LRV of about 5 or above can be reached.

ACKNOWLEDGMENT

Funding from Teva Pharmaceuticals is gratefully acknowledged.

AUTHOR CONTRIBUTIONS

Rong Fan conducted most of the filtration experiments and data analysis. Fnu Namila performed some filtration experiments and virus assay as well as helped write the first draft of the manuscript. Davar Sansongko did most of the virus assay. S. R. Wickramasinghe involved in the funding acquisition, conceptualization, and editing of the manuscript. Mi Jin involved in funding acquisition and project conceptualization. Dharmesh Kanani involved in funding acquisition, conceptualization, project administration, critical review, and editing of the manuscript. Xianghong Qian involved in the funding acquisition, conceptualization, project supervision, data analysis, writing the first draft, editing, and finalizing the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author on reasonable request.

ORCID

Xianghong Qian http://orcid.org/0000-0001-5619-9782

REFERENCES

Asher, D. R., Katz, A. B., Khan, N. Z., & Mehta, U. (2017). *Methods of producing high titer, high purity virus stocks and methods of use thereof*. Google Patents.

Bohonak, D. M., Mehta, U., Weiss, E. R., & Voyta, G. (2020). Adapting virus filtration to enable intensified and continuous monoclonal antibody processing. *Biotechnology Progress*, e3088.

Bolton, G., Cabatingan, M., Rubino, M., Lute, S., Brorson, K., & Bailey, M. (2005). Normal-flow virus filtration: Detection and assessment of the endpoint in bioprocessing. *Biotechnology and Applied Biochemistry*, *42*(2), 133–142. https://doi.org/10.1042/ba20050056

CPMP, I. (1997). *Q5A. Note for guidance on quality of biotechnological products: Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin*. (CPMP/ICH/295/95). http://www.emea.eu.int

David, L., Niklas, J., Budde, B., Lobedann, M., & Schembecker, G. (2019). Continuous viral filtration for the production of monoclonal antibodies. *Chemical Engineering Research and Design*, *152*, 336–347.

Dishari, S. K., Venkiteshwaran, A., & Zydney, A. L. (2015). Probing effects of pressure release on virus capture during virus filtration using confocal microscopy. *Biotechnology and Bioengineering*, *112*(10), 2115–2122.

Dougherty, & R. (1964). In R. J. C. Harris (Ed.), *Techniques in experimental virology*. Academic Press.

Gavasane, M., Ali, A. U., Samagod, A., Kaundinya, J., Daftary, G., Preuss, T., & Banerjee, S. (2013). Process development and spiking studies for virus filtration of r-hFSH. *BioPharm International*, *26*(5).

Giglia, S., Bohonak, D., Greenhalgh, P., & Leahy, A. (2015). Measurement of pore size distribution and prediction of membrane filter virus retention using liquid–liquid porometry. *Journal of Membrane Science*, *476*, 399–409.

Guideline, I. H. T. (1997). Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin Q5A (R1). *Current Step*, *4*.

Hongo-Hirasaki, T., Komuro, M., & Ide, S. (2010). Effect of antibody solution conditions on filter performance for virus removal filter Planova™ 20N. *Biotechnology Progress*, *26*(4), 1080–1087.

Kozlov, M., Cataldo, W., Potty, A., Galipeau, K., Hamzik, J., Umana, J., & Peeck, L. (2018). *Removal of protein aggregates from biopharmaceutical preparations in a flow-through mode*. Google Patents.

LaCasse, D., Genest, P., Pizzelli, K., Greenhalgh, P., Mullin, L., & Slocum, A. (2013). Impact of process interruption on virus retention of small-virus filters. *Bioprocess International*, *11*(10), 34–44.

LaCasse, D., Lute, S., Fiadeiro, M., Basha, J., Stork, M., Brorson, K., Godavarti, R., & Gallo, C. (2016). Mechanistic failure mode investigation and resolution of parvovirus retentive filters. *Biotechnology Progress*, *32*(4), 959–970.

Lute, S., Bailey, M., Combs, J., Sukumar, M., & Brorson, K. (2007). Phage passage after extended processing in small-virus-retentive filters. *Biotechnology and Applied Biochemistry*, *47*(3), 141–151.

Namila, F., Zhang, D., Traylor, S., Nguyen, T., Singh, N., Wickramasinghe, R., & Qian, X. (2019). The effects of buffer condition on the fouling behavior of MVM virus filtration of an Fc-fusion protein. *Biotechnology and Bioengineering*, *116*(10), 2621–2631.

Shamashkin, M., Godavarti, R., Iskra, T., & Coffman, J. (2013). A tandem laboratory scale protein purification process using Protein A affinity and anion exchange chromatography operated in a weak partitioning mode. *Biotechnology and Bioengineering*, *110*(10), 2655–2663.

Sofer, G., Brorson, K., Abujoub, A., Aranha, H., Burnouf, T., Carter, J., & Krishnan, M. (2005). PDA Technical Report No. 41: Virus filtration. *PDA Journal of Pharmaceutical Science and Technology*, *59*(Suppl 2), 1–42.

Strauss, D., Goldstein, J., Hongo-Hirasaki, T., Yokoyama, Y., Hirotomi, N., Miyabayashi, T., & Vacante, D. (2017). Characterizing the impact of pressure on virus filtration processes and establishing design spaces to ensure effective parvovirus removal. *Biotechnology Progress*, *33*(5), 1294–1302.

Stuckey, J., Strauss, D., Venkiteshwaran, A., Gao, J., Luo, W., Quertinmont, M., O'Donnell, S., & Chen, D. (2014). A novel approach to achieving modular retrovirus clearance for a parvovirus filter. *Biotechnology Progress*, *30*(1), 79–85.

Wickramasinghe, S. R., Stump, E. D., Grzenia, D. L., Husson, S. M., & Pellegrino, J. (2010). Understanding virus filtration membrane performance. *Journal of Membrane Science*, *365*(1–2), 160–169.

Willkommen, H., BluMel, J., Brorson, K., Chen, D., Chen, Q., GroNer, A., Kreil, T. R., Robertson, J. S., Ruffing, M., & Ruiz, S. (2013). Meeting Report—Workshop on virus removal by filtration: Trends and new developments. *PDA Journal of Pharmaceutical Science and Technology*, *67*(2), 98–104.

Woods, M. A., & Zydney, A. L. (2014). Effects of a pressure release on virus retention with the Ultipor DV20 membrane. *Biotechnology and Bioengineering*, *111*(3), 545–551.

Yamamoto, A., Hongo-Hirasaki, T., Uchi, Y., Hayashida, H., & Nagoya, F. (2014). Effect of hydrodynamic forces on virus removal capability of Planova™ filters. *AIChE Journal*, *60*(6), 2286–2297.

Zydney, A. L. (2016). Continuous downstream processing for high value biological products: A review. *Biotechnology and Bioengineering*, *113*(3), 465–475.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Fan, R., Namila, F., Sansongko, D., Wickramasinghe, S. R., Jin, M., Kanani, D., & Qian, X. (2021). The effects of flux on the clearance of minute virus of mice during constant flux virus filtration. *Biotechnology Bioengineering*, 118, 3511–3521. https://doi.org/10.1002/bit.27778